REMARKS

Rejection of claims under 35 U.S.C. 112:

Claims 7-11 and 19-28 have been rejected under §112, second paragraph. The Action states that the metes and bounds of the term "physiological condition" are not defined. Applicants respectfully disagree.

In the Specification, on page 19, lines 12-17 the term "physiological condition" is defined.

Claims 7-11 and 19-28 have been rejected under §112, first paragraph for containing the limitation of "under physiologic conditions" as subject matter which was not described in the specification. Applicants refer to page 19, lines 12-17 for its specific reference to the definition.

Applicants request that the rejections be removed.

Rejection of claims under 35 U.S.C. 102:

Claims 7-11 and 19-28 have been rejected under §102(b) as being anticipated by the Pierce catalog and Arpicco *et al.* Applicants have canceled claims 19-23 and amended claims 7 and 24 to be consistent with the telephone interview and to obviate the rejection.

In the proposed claim, we have added the limitation of two reactable groups, at least one on each side of the disulfide bond that have reacted to form a covalent bond with one or more molecules, because as written the current claim covered the prior art in the Pierce catalogue. The Pierce examples teach disulfide cross-linkers that contain the pyridyl dithio linkage (pyridine thiol), which would be physiologically labile if injected into a cell (just as a note; two similar compounds, Alderthiol (-2 and –4), are the disulfides of pyridine thiols, and are sold by Aldrich Chemical Company). However, this teaching is different than our invention in that this cross-linker reacts via a cleavage of the disulfide bond in the pyridyl disulfide upon exposure to a free thiol (Figure 1). The resulting reaction results in the formation of a new disulfide bond (which may or may not be physiologically labile under our specification, depending on the structure of the free thiol) and pyridine-2-thione.

Figure 1. Reaction of a Pyridyl Disulfide Cross-linker

Our invention teaches physiologically labile disulfide cross-linkers in which the reactive groups are not the disulfide bond. As outlined in the specification, we have invented cross-linkers for in vivo and in vitro use that have bifunctional (hetero or homo) groups for reacting with other molecules, leaving the original disulfide bond of the cross-linker intact. Included in our invention is the pyridine thiol system, due to the inherent lability of the disulfide bonds obtained from this system. However, in contrast to the systems taught in Pierce, we have developed systems in which the disulfide bond is not the reactive group. We teach systems including other functional groups on the ring, for example an N-hydroxy succinimidyl ester, for reacting with an amine on another molecule. This results in the formation of a new molecule that still contains the labile disulfide from the pyridine thiol group.

As we have discussed during the prosecution of this application, the stability of disulfide bonds (thermodynamics) and the rate (kinetics) of disulfide reduction are related to the pKa of the respective thiols. Glutathione for example has a pKa of 8.9. The pyridine thiols have lower pKa's, and therefore one would expect a disulfide bond constructed from a pyridine thiol would be cleaved more rapidly than oxidized glutathione (the disulfide of glutathione) (Figure 2, top number is the pKa listed for the thiol). However, we are not aware of any disulfide prepared with an additional functional group linked to the pyridine ring as described in our invention. Additionally, we have not found an example described in the literature in which a disulfide has been described with the pyridine nitrogen bonded to an atom other than the pyridine ring carbons (for example an acylation or alkylation reaction). The only known compounds we are aware of in which, for example, the pyridine nitrogen is alkylated, exist as the thione, and not the disulfide (Figure 1). This is due to the very low pKa of the pyridine-2-thiol, indicating that the system prefers to put the electron density on the pyridine nitrogen. Any reaction of the pyridine nitrogen would therefore only increase this electron flux away from the thiol, dramatically decreasing any the stability of any disulfide bond to a point where one would not be able to isolate it.

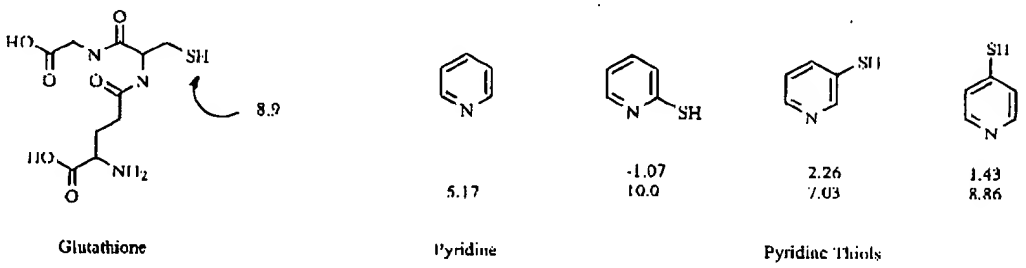

Figure 2. pKa's of Pyridine Thiols

We have based our benchmark on the physiological reducing agent glutathione (pKa of glutathione is 8.9, Figure 3). Glutathione is an N-acyl cysteine amide. The thiol pKa of the parent cysteine is 8.3 (Handbook of Biochemistry and Molecular Biology). Acylating the cysteine nitrogen increases the pKa of the thiol to 9.5 (penicillamine, a dimethyl derivative shows a similar trend, Figure 3). Amidation or esterification of the cysteine carboxylic acid on the other

hand, lowers the pKa of the thiol (6.7 for cysteine ethyl ester, Figure 3). As another example, for cysteine-cysteine, the pKa of the thiol on the N-terminal amino acid is 7.27, whereas the pKa of the C-terminus cysteine thiol is 9.35 again indicating that amidation of the cysteine carboxylic acid decreases the pKa of the thiol (relative to cysteine), and acylation of the cysteine nitrogen causes the pKa of the thiol to increase (Figure 3). Combining both substitutions would result in an N-acyl cysteine amide (or ester), with a resultant pKa similar to that of glutathione or other protein.

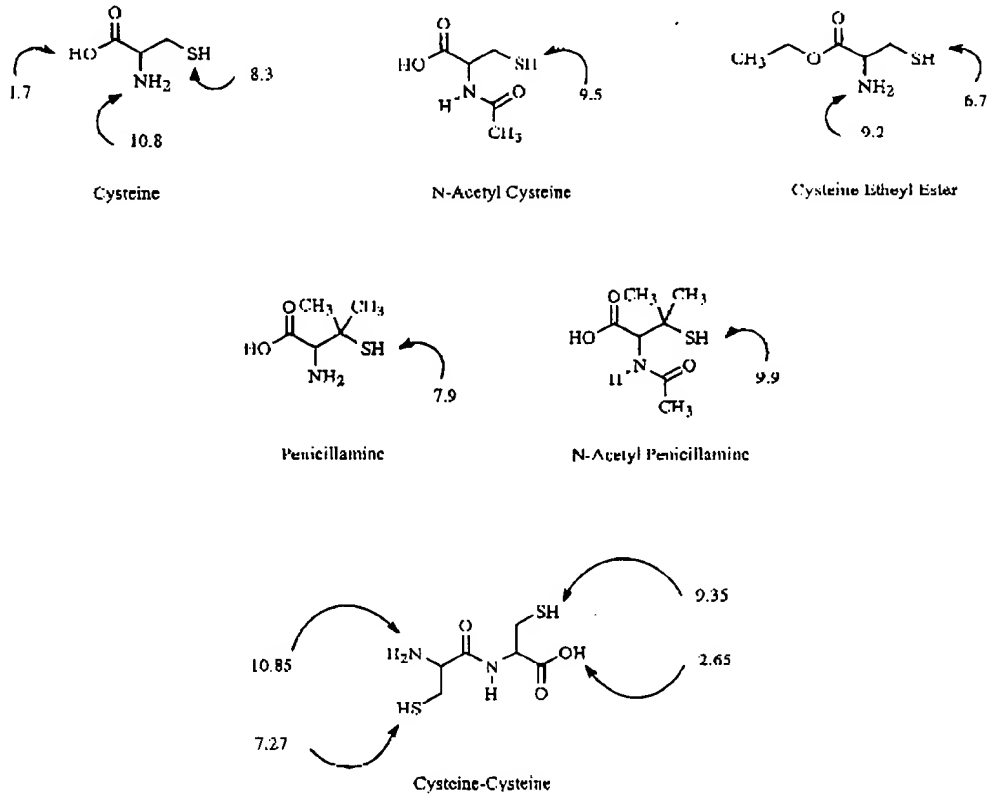

Figure 3. pKa's For Cysteine Thiols

Another point that arose during our recent discussion involves the effect of additional reactive groups such as amines or other thiols or some other withdrawing group, on the disulfide. Generally, substituents that affect a neighboring group (inductive and field effects) are either close to the neighboring group (1-3 bonds away), or are conjugated to the neighboring group via multiple bonds (sp, sp2, and/or aromatic rings). The preceding discussion gives several examples of these effects. In the absence of conjugation, substitution at more distant atoms has much less effect on pKa than substitution close to the thiol.

In order to show longer-range effects, a series of terminal cysteine peptides has been described in the literature. The pKa for the thiol of the base amino acid is 8.3 (Figure 4). The corresponding pKa for the peptide cys-gly-gly has been determined to be 6.36, again showing a decrease in the pKa following amidation. Increasing the length of the peptide with by an additional two glycine residues had little effect on the pKa of the cysteine thiol, which was measured at 6.01 (Figure 4). Although the direction of the pKa change is predictable, following modification of the base amino acid, the magnitude of this change is more uncertain. For example in comparing the cys-cys system (Figure 3) and the cys-gly-gly peptides (Figure 4), the glycine systems have lower thiol pKa's. This difference indicates the complexity of these systems, where one group affects another, however, distinct trends emerge, and can be of predictive power.

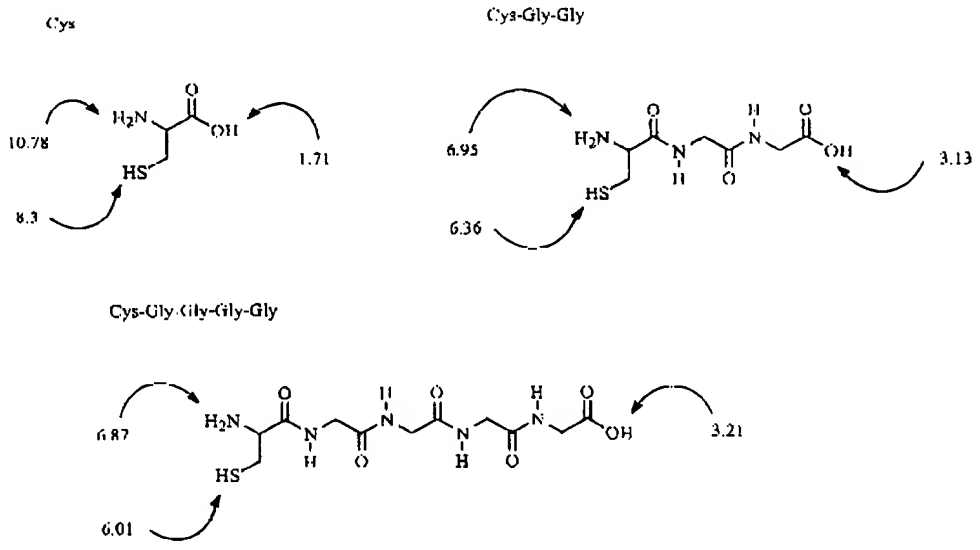

Figure 4. pKa's of Cysteine Peptides

In reviewing these trends, it is apparent that the groups in close proximity (1-3 bonds) to the thiol have the greatest effect on the thiol pKa. In all cases in which the cysteine carboxylic acid is reacted with either an amine or an alcohol (to form an amide or an ester), in which the cysteine amine is free, the pKa of the thiol is lower than that of glutathione. One of the smallest decreases in thiol pKa that we are aware of following amidation is the cys-cys system described in figure 3, with a pKa decrease of 1 unit. Conversely, in all cases in which the amine is acylated (as in a peptide) the thiol pKa is greater than that of glutathione. Once again, in all examples that we are aware of, this increase is greater than one pKa unit, and generally more.

On the other hand, longer-range effects have a much lower influence on the magnitude of the thiol pKa. For example, when the carboxylic acid is moved from 9 to 15 atoms from the thiol,

the pKa of the thiol is only 0.35 units lower (Figure 4). Similarly, the pKa values for the other applicable atoms within the molecule show very minor changes in the pKa's.

Double Patenting:

Applicants have canceled claims 19-23 to obviate the double patenting rejection.

The Examiner's objections and rejections are believed to be overcome by this response to the Office Action. In view of Applicants' amendments and discussion, it is submitted that independent claims 7 and 23 are allowable and therefore dependent claims 8-11 and 25-28, which depend either directly or indirectly from the independent claims, should be allowable as well. Applicants respectfully request an early notice to such effect.

Respectfully submitted,

Mark K. Johnson, Reg. No. 35,909
Mirus
505 South Rosa Road
Madison, WI 53719
608.238.4400

I hereby certify that this correspondence is being sent by facsimile transmission to: Commissioner for Patents, Washington, DC 20231, CM1 Fax Center 703.308.4242 on Friday, October 04, 2002.

Mark K. Johnson